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13. ABSTRACT (Maximum 200 words) The biodegradation characteristics of three fuel system icing inhibitors (FSII) were evaluated. FSII are jet fuel additives that partition into water readily and are present in the water drained from storage tank bottoms in concentrations approaching 40%. These concentrations raise concerns as to the disposal and handling of these wastes. The current FSII, DiEGME was evaluated along with two new candidates, dipropylene glycol and glycerol formal. DiEGME appeared to be moderately but not completely biodegradable. It is likely that much of it would be removed in a wastewater treatment plant. Dipropylene glycol only showed signs of degradation after more than three weeks at which point it degraded moderately well. The third FSII, glycerol formal did not show any signs of biodegradability during the five week period of testing. Preliminary toxicity and inhibitory tests were carried out for these chemicals at high and low concentrations. DiEGME appeared to be most toxic to microorganisms at high concentrations, dipropylene glycol show moderate toxicity, and glycerol formal showed little. At low concentrations, none of the chemicals appeared to inhibit the activity of microorganisms.				
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Biodegradability and Microbial Toxicity of Aircraft Fuel System Icing Inhibitors

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ABSTRACT: In a pollution prevention and chemical substitution effort, the U.S. Air Force and Navy formed a joint initiative to find safer, more environmentally acceptable jet fuel system icing inhibitors (FSII) for military aircraft. Standard biochemical oxygen demand (BOD) analysis and variations of the BOD procedure were used as simple screening tools to evaluate the potential for aquatic biodegradation and microbial toxicity of proposed FSIs. This laboratory evaluation of biological properties allows prediction of the biotreatability of the chemicals in wastewater treatment plants, and their potential application as biocides at higher concentrations. The current FSII, diethylene glycol monomethyl ether (DiEGME) was evaluated along with two new candidate compounds, dipropylene glycol and glycerol formal. At a low concentration (3.5 mg/L), DiEGME exerted a BOD₅ of about 27% of theoretical oxygen demand. Test concentrations of ≥ 7 mg/L had decreasing oxygen consumption rate and extent, typical of a material with potential aquatic microbial toxicity. Dipropylene glycol began to moderately degrade only after more than 3 weeks exposure to microorganisms obtained from raw sewage. Glycerol formal showed no signs of biodegradation during a 5-week test period. In a simple microbial toxicity test DiEGME was most toxic, dipropylene glycol was moderately toxic, and glycerol formal showed little toxicity. At low concentrations (7 mg/L), none of the chemicals significantly inhibited microbial activity ($P = 0.34$). © 1999 by John Wiley & Sons, Inc. *Environ Toxicol* 14: 383–390, 1999

Keywords: toxicity; BOD; diethylene glycol monomethyl ether; dipropylene glycol; glycerol formal; biodegradability

INTRODUCTION

Pollution prevention initiatives have resulted from the increased need to protect human health and the environment while minimizing future waste disposal costs. One of the armed forces' objectives has been a search for more environmentally friendly fuel system icing inhibitors (FSII). To prevent water dissolved in jet fuel from forming filter-clogging ice crystals, commercial aircraft employ fuel filter heaters. Military aircraft are

not equipped with such devices as they are too bulky and heavy for use on aircraft in which top performance is critical (Goodger, 1995). FSIs are fuel additives that depress the freezing point of the dissolved water. At high concentrations, the additives may also serve as biocides, preventing microbial growth in fuel storage tanks that foul or corrode tanks and fuel systems. Effective FSIs have very high water-fuel partitioning ratios, causing the water accumulating at the bottom of jet fuel storage tanks (tank bottoms) to have concentrations of FSII ranging from 20 to 50%. These tank bottoms are regularly drained from jet fuel storage

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tanks. The potentially high concentrations of FSII in the wastewater raise concerns as to the handling and disposal of these wastes.

Ethylene glycol monomethyl ether (EGME) was used as a FSII for many years. It was replaced by diethylene glycol monomethyl ether (DiEGME) because of concern over potential environmental toxicity. The aquatic toxicity of oxyethylene molecules is inversely related to chain length, longer chains being less toxic. Nevertheless, concerns about the potential environmental impact of DiEGME have driven the search for an even less toxic, more environmentally friendly material (Bridie, et al., 1979; Schuurman, 1990; Mushrush et al., 1997). Although DiEGME has been shown to be degradable in wastewater treatment systems, concentrations found in tank bottoms may be high enough to disrupt the microbiological treatment process. The very high oxygen demand of these concentrated wastewaters may overtax the ability of the aeration system of a wastewater treatment plant and disrupt the treatment process.

Ideal characteristics of a FSII in fuels and tank bottoms conflict with desirable characteristics for materials to be treated in wastewater treatment systems. A material toxic enough to act as a biocide in a tank bottom may not be degradable enough to be removed by a wastewater treatment system. Ready degradability is often accompanied by relatively high oxygen demand that can overwhelm the oxygen supply capabilities of a wastewater treatment plant.

The first step in the initiative to find a substitute for the current FSII involved selection of candidate chemicals using quantitative structure activity relationship (QSAR) computer software to predict partitioning behavior and toxicity. Commercially available chemicals were purchased for testing; those chemicals not otherwise available were to be synthesized. Fuel/water partitioning ratios were measured in the laboratory, and promising candidates were tested on a bench scale for their ability to inhibit ice crystal formation in jet fuel. Chemicals that successfully inhibited ice crystal formation were further tested for biodegradability and toxicity, the scope of this study.

Several simple, low-cost screening tests were used to determine the relative aerobic biodegradability and toxicity of candidate FSII. The tests were used to determine (1) the likely treatability of the compounds in industrial wastewater treatment plants (IWTP), and, (2) the potential toxicity of the chemicals to microorganisms, either in IWTPs, or as biocides in tank bottoms (a function fulfilled by the current FSII, DiEGME).

The tests to be used for screening the candidate FSII should (1) be comparative and reproducible, (2) cost relatively little, and (3) require a minimal amount

of test chemical, as availability of the candidate chemicals was expected to be constrained.

MATERIALS AND METHODS

Test Chemicals

Although several novel chemicals were identified as candidate FSII in the first review, the only three chemicals to reach the laboratory screening stage were commercially available. The chemicals are currently used in the pharmaceutical and chemical manufacturing industries as solvents or feedstocks (Budavari, 1996). The three chemicals screened for biodegradability in the tests reported here were the current FSII, diethylene glycol monomethyl ether (DiEGME), and the candidate compounds dipropylene glycol (DPG) and glycerol formal (GF) (Fig. 1). Physical and chemical characteristics of the chemicals are shown in Table I.

BOD₅

Aerobic biodegradability of the proposed FSII, as measured by the standard 5-day biochemical oxygen demand test (BOD₅), provides an estimation of the likely treatability of these wastes in a wastewater treatment system (APHA, 1995). The concentrations of FSII appropriate for the BOD₅ test were of the same order of magnitude as the concentrations expected during typical operation of an aeration basin of a military installations' IWTP (25–50 mg/L). The high water solubility of FSII also contributed to the selection of a closed-bottle test since constant stirring during incubation would not be necessary. The tests followed the standardized procedures outlined in *Standard Methods*

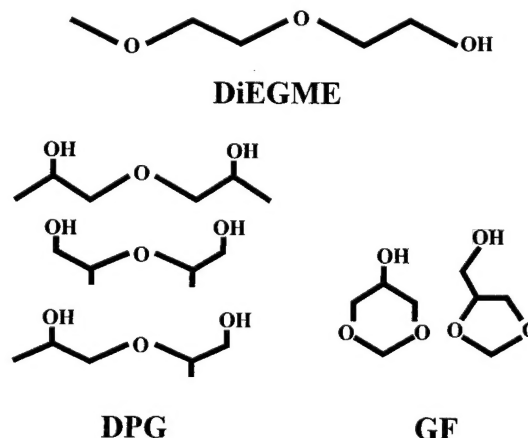


Fig. 1. Molecular structure of fuel icing inhibitor test materials. DiEGME has only one molecular structure, GF has two isomers, and DPG has three isomers.

TABLE I. Physical and chemical characteristics of test compounds

Compound	Molecular Weight	Specific Gravity	Water/Fuel Partition Ratio	CAS Number	Composition	Source
DiEGME	120.15	1.054	480	111-77-3	C ₅ H ₁₂ O ₃	Aldrich ^a
DPG	134.18	1.0224	> 600	25265-71-8	C ₆ H ₁₄ O ₃	Arco ^b
GF	104.11	1.203	570	86687-05-0	C ₄ H ₈ O ₃	Aldrich ^a

^a Aldrich Chemical Co., Inc. Milwaukee, WI.^b Arco Chemical Company, Newton Square, PA.

for the Examination of Water and Wastewater, including using 300 mL BOD bottles and incubation at 20°C for 5 days in the dark (APHA, 1995). A dissolved oxygen probe was used to measure O₂ levels at the beginning and end of the incubation period.

Supernatant from settled raw sewage was used as the microbial seed since it was biologically diverse but tended to have a minimal population of nitrifying bacteria. The presence of nitrifiers complicated the interpretation of oxygen uptake data since they oxidize ammonia, present in the buffer solution, to nitrate. All BOD₅ tests were run in triplicate.

Glucose/glutamic acid standard check samples (300 mg/L) were run with each set of BOD₅ analyses as a quality control measure. Over the course of the study, the average BOD₅ of these samples was 185 ± 22 mg/L, comparable to the levels reported in the interlaboratory study described in *Standard Methods* (198 ± 30.5 mg/L) (APHA, 1995).

BOD is an empirical test. To be considered valid, *Standard Methods* (APHA, 1995) requires that at the end of 5 days incubation, test dilutions consume at least 2 mg/L O₂ and have a residual level of O₂ of at least 1 mg/L. Dilution water should consume less than 0.2 mg/L O₂. These rigorous standards of performance are met only by carefully controlled laboratory procedures. Test solutions that are too strong or too weak produce residual O₂ levels outside the acceptable range.

A simple classification scheme for biodegradability using the BOD₅/COD (chemical oxygen demand) ratio is presented by Lyman et al. (1990). They classified chemicals with a BOD₅/COD ratio of < 1% as "relatively undegradable." Chemicals with a ratio between 1 and 10% were called "moderately degradable," and chemicals with ratios > 10% were considered "relatively degradable." Since COD information was not available for all the compounds, theoretical oxygen demand (ThOD) values were substituted, leading to a slightly more conservative estimate of biodegradability.

Long-Term Studies

In order to evaluate the kinetics of the biodegradation of these compounds, two additional tests were em-

ployed. First, a long-term (37-day) BOD test using six replicates, 300 mL bottles, and manual reaeration, followed the guidelines of *Standard Methods* proposed ultimate BOD test (APHA, 1995). Second, a Columbus Instruments Micro-Oxymax automatic closed-loop respirometer was used as an additional check of biodegradability. Microcosms for the respirometer were constructed using 1 L bottles in which 800 mL of buffered BOD dilution water, 4 mL of settled raw sewage supernatant, and the test chemical were added and incubated at 30°C. The respirometer sampled the oxygen concentration of the air in the headspace of each bottle automatically at regular, set intervals and refreshed this headspace as necessary. Two replicate microcosms were used for each potential FSII, along with a seeded dilution water blank.

Inhibition of Respiration

Some compounds, at certain concentrations, may exert no measurable oxygen demand. This precludes measuring toxicity by changes in relative oxygen demand with changing concentrations. A novel solution to this problem was to test the standard BOD glucose/glutamic acid check solution along with the candidate compounds. A mixture of glucose and glutamic acid (150 mg/L each) produces an expected BOD₅ of about 200 mg/L. Note that this mixture of readily degradable compounds, used as the standard in BOD analyses, produces an oxygen demand of only about 66% of the theoretical value (APHA, 1995). This level of oxygen demand, or BOD₅, is typical of the expected demand of domestic wastewaters (Tchobanoglous and Burton, 1991).

Acclimated Seed Tests

An effort was made to acclimate microbial cultures to the test compounds for use as seed in the BOD tests. Flasks (1L) with wastewater supernatant were continuously aerated while approximately 50 mg/L of the test materials were added every other day. Inorganic nutri-

ents from BOD dilution water buffer solution were added twice weekly. Yeast extract (25 mg/L) was added to enhance initial microbial growth. Cultures were grown for 3 weeks before BOD testing was attempted. Cultures were not provided additional FSII for 4–7 days before testing to allow consumption of residual test materials.

Applicability as a Biostat

Selecting an appropriate screening test for the applicability of each FSII as a biostat for the tank bottoms was challenging as the concentrations typically found in tank bottoms were over 20% and not enough FSII was expected to be available to conduct closed bottle tests in this concentration range. An agar diffusion test was used as a preliminary screening method since high-concentration toxicity can be evaluated using relatively small amounts of chemical (Mills, 1996). In this test, 0.1 mL of FSII was added to a well excavated into the surface of a nutrient agar plate. As the material diffused into the agar, a concentration gradient was produced, with high concentrations near the well and lower concentrations away from the well.

The surfaces of the test plates were inoculated with a sewage supernatant microbial seed and allowed to grow overnight. In control plates, microorganisms produced an even layer of growth across the surface of the agar. In plates with test compounds that exhibit toxicity, a clear zone of growth inhibition was observed around the well. The clear area was proportional to diffusivity and toxicity of the test materials. For compounds that are toxic at high concentrations, but readily degradable at lower concentrations, microbial growth beyond the clear zone is often significantly enhanced.

This produces a halo effect with more dense microbial growth in the gradient beyond the toxic concentration.

RESULTS AND DISCUSSION

Biodegradation

For the BOD test results summarized in Table II, only the DiEGME results strictly met the O_2 consumption and residual requirements. Nevertheless, results that fall slightly out of the acceptable range can provide useful information when viewed in the context of a broad series of tests. Such results will be presented in the following discussions.

Using the degradability classification of Lyman et al. (1990), from the results shown in Table II, DiEGME would be classified as moderately degradable and the two other compounds would be considered relatively undegradable.

BOD₅

Valid, reproducible test results were obtained for DiEGME. The BOD₅ results, presented as a fraction of ThOD (Fig. 2), varied with test concentration. At the lowest concentration tested (3.5 mg/L), DiEGME appeared to be moderately degradable (> 25% of ThOD). However, the measured BOD₅ decreased as the test concentration increased, a strong indication of microbial toxicity at higher concentrations.

The BOD₅ exerted by GF represented less than 0.5% of the Theoretical Oxygen Demand (ThOD), although the low oxygen consumption allowed valid BOD₅ computations only when the test concentration was

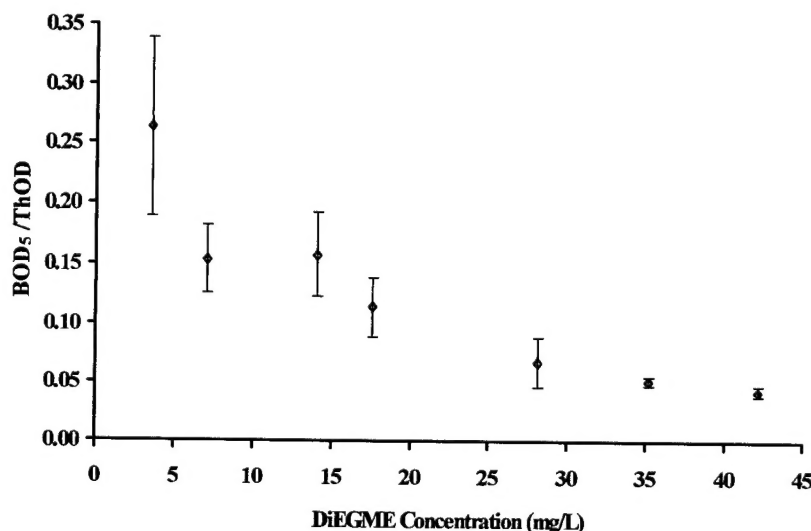


Fig. 2. DiEGME BOD₅/theoretical oxygen demand ratio.

above 200 mg/L (Fig. 3). The GF was 98.8% pure and it is possible that the small, but consistent, measurable oxygen consumption was the result of degradation of the unidentified impurities. DPG exerted no measurable BOD_5 over a wide range of test concentrations (Fig. 3).

Long-Term BOD

In a long-term BOD test (37 days, Fig. 4) DiEGME was moderately degradable, showing a fairly rapid rate of oxygen uptake over the first 15 days of the test, with a more gradual consumption rate later in the test period.

The DPG samples showed no signs of degradation until after 25-days exposure to the microorganisms, then degradation proceeded relatively rapidly to over 30% of the ThOD by the end of the test period. Throughout the test, the GF samples showed no evidence of degradation at the low concentration tested (7 mg/L). The negative numbers in Fig. 4, and the apparent decrease in demand for DiEGME after day 14, are artifacts resulting from subtraction of oxygen uptake by the dilution water blanks. At these low levels of oxygen consumption, some unavoidable noise is introduced into the analyses. Nitrification is likely one cause of the variability.

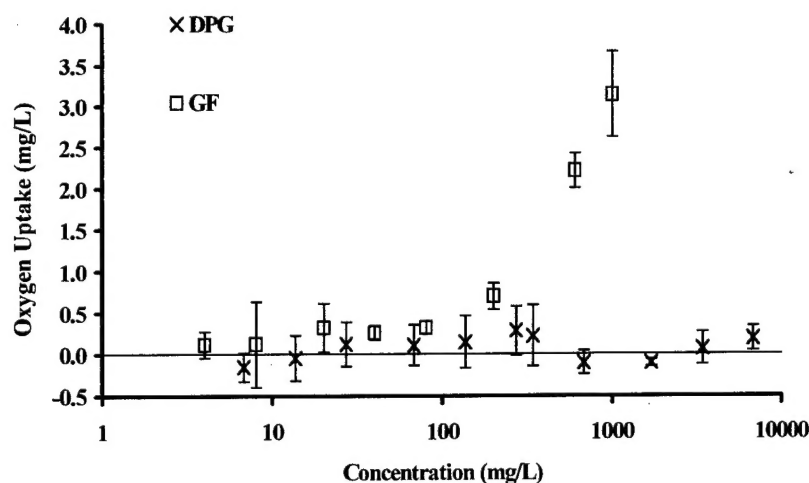


Fig. 3. Oxygen uptake of DPG and GF at various substrate concentrations. Negative numbers are the result of subtraction of blank dilution water values.

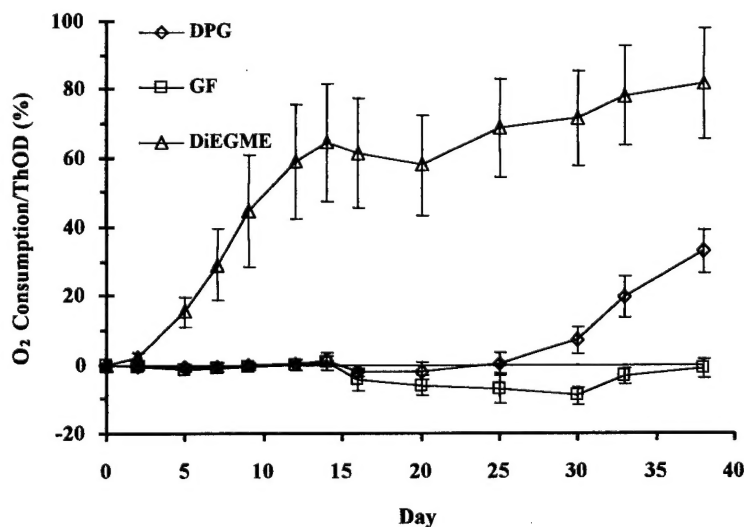


Fig. 4. Long-term biochemical oxygen demand for three FSIs. All test concentrations were 7.0 mg/L. Data points are the average of four to six replicates.

Mushrush et al. (1997) calculated an environmental half-life for DiEGME of 2–16 days, based on a model estimator of BOD. The average BOD₅ measured in our laboratory for DiEGME, 8.9% of ThOD (Table II), suggested a significantly longer half-life, perhaps more than double the period estimated by the modeling studies. However, the longer term empirical tests suggested a half-life of just over 10 days (Fig. 4) in general agreement with Mushrush et al. (1997). The DiEGME concentration tested (7 mg/L) reached about 80% of the ThOD by the end of the 37-day test.

The effort to determine BOD using an acclimated microbial culture as seed was not successful. Although oxygen consumption for the glucose/glutamic acid check samples were at acceptable levels, oxygen consumption for the FSII test solutions were below the values determined with an unacclimated seed. However, an interesting observation merits further exami-

nation. The DPG culture produced relatively few planktonic organisms, but showed substantial attached growth on the walls of the culture flask. This suggests that microorganisms growing in a biofilm may be more efficient than planktonic microorganisms in degrading this material, perhaps suggesting a treatment method for DPG wastewater.

The closed-loop respirometer tests, conducted at a higher temperature than the BOD tests (30 vs 20°C), confirmed the results of the long-term closed-bottle biodegradation tests (Fig. 5). The 10°C temperature increase would be expected to accelerate, perhaps double the degradation rate of the degradable compounds. Only DiEGME, tested at two concentrations, showed measurable respiration above that of the seed control (blank) in the 6 days of the test. Headspace gas analyses, made at approximately 2.6 h intervals, provided a higher resolution look at oxygen consumption than

TABLE II. Biochemical oxygen demand (BOD) test results

Compound	ThOD (g O ₂ /g)	COD (g/g)	BOD ₅ (g/g)		BOD ₅ (% ThOD)	Test Concentration Range (mg/L)
			Prior Studies	This Study		
DiEGME	1.73	1.71	0.12 ^a	0.16 ± 0.09	8.9 ± 5.4	14–1400
DPG	1.91	1.84	0.09 ^a	< 0.001 ^c	< 0.06 ^a	14–6816
GF	1.38	N/A	None	0.0038	0.3	4–1003
G/GA (Standard)	1.045	N/A	0.66 ± 0.097 ^b	0.59 ± 0.070	56.5	300

^a Bridie et al., 1979.

^b APHA, 1995.

^c No valid results obtained, insufficient O₂ consumption.

N/A: not available.

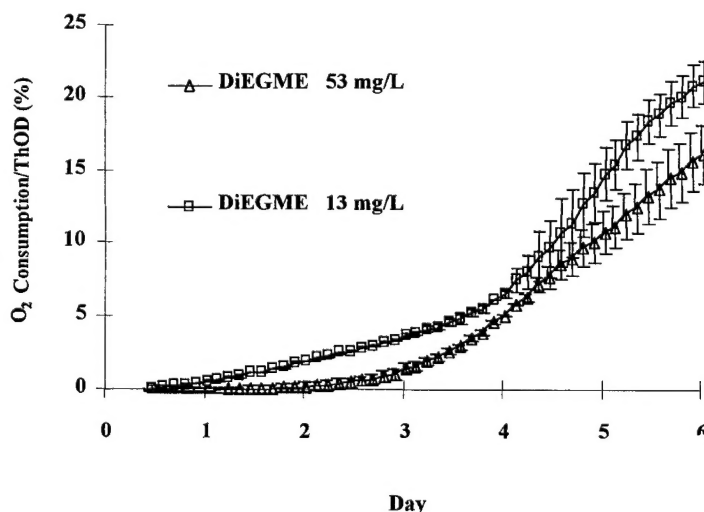


Fig. 5. Oxygen consumption for two concentrations of DiEGME, as measured by a respirometer. Points represent average net consumption from two microcosms (seed consumption subtracted).

could be made with traditional BOD measurements. As with the traditional BOD tests, however, measurable oxygen consumption did not occur until the second day of exposure for the higher concentration (53 mg/L). The low concentration (13 mg/L) showed consumption above seed-control levels before the end of the first day of testing. Although the test was halted at 6 days because of temperature control problems in the laboratory, the lower test concentration achieved over 20% of the ThOD and the higher concentration was at about 16% of ThOD. These results also suggested some toxicity at higher DiEGME concentrations.

A second long-term degradation test, using only DiEGME at various concentrations, was completed to help clarify the mechanisms of the degradation process of this compound (Table III). Note that for the low concentrations of DiEGME (3.5 mg/L), about the same amount of oxygen was consumed in the second 5-day period as in the first. Likewise, for the 7 mg/L test concentration, nearly equal amounts were consumed in the two periods, but at only about one-half the level of consumption observed in the 3.5 mg/L sample. For the 14 mg/L samples, more oxygen demand was exerted in the second 5-day period. This suggests that at 3.5 mg/L, a sufficient microbial population already existed in the seed to perform degradation. For 14 mg/L, microbial growth or enzyme production was necessary to fully use the substrate. By the end of the 20-day test period, the total relative oxygen consumption was nearly identical for the 7 and 14 mg/L tests, and markedly higher for the 3.5 mg/L test. Again, this supports the suggestion of ready degradability at low concentrations and microbial inhibition at higher concentrations for DiEGME.

Toxicity to Biological Treatment Works

A simple check for toxicity can be performed by comparing oxygen consumption ascertained from the BOD tests described above as a function of FSII concentration. A decrease in relative oxygen consumption with increased FSII concentration would be evidence of

toxicity (Pitter and Chudoba, 1990; Wetzel and Murphy, 1991; APHA, 1995).

The results shown in Fig. 2 suggest microbial toxicity with increasing concentrations of DiEGME. The BOD₅ inhibition test (Table IV) used the standard glucose/glutamic acid test mixture to provide a consistent, known oxygen demand. A decrease in this demand in the presence of the FSII would suggest toxicity. Since DiEGME exerted a measurable BOD₅, the calculated oxygen consumption associated with the biodegradation of the DiEGME was subtracted from the total oxygen consumption before a BOD was calculated in these samples. Although the variability of the triplicate samples was increased in the DiEGME tests, an analysis of variance suggested that there was no statistically significant difference (P value = 0.34) in the final BODs, with or without the FSII, indicating no toxicity at the tested FSII concentration of 7 mg/L.

Tank Bottom Biostat

The agar diffusion toxicity test showed that, after approximately 30 h of incubation at 30°C, DiEGME produced large circles of inhibition, devoid of visible microbial growth. This clear zone, however, was surrounded by a halo of more dense growth, suggesting that at the lower concentrations resulting from diffusion, microbial growth was enhanced. DPG produced slightly smaller circles of inhibition and some visible growth appeared in the zone. No area of enhanced growth was observed. GF had minimal inhibitory ef-

TABLE IV. Inhibition test results

Test Chemical	Concentration (mg/L) ^a	BOD (mg/L)
Glucose/glutamic acid (g/g) control	300	177 ± 1
DiEGME + g/g	7 + 300	236 ± 83
Dipropylene glycol + g/g	7 + 300	179 ± 7
Glycerol formal + g/g	7 + 300	189 ± 10

^a Test concentrations included 300 mg/L from glucose/glutamic acid and 7 mg/L from the FSII.

TABLE III. BOD/ThOD ratios (in percent) for DiEGME

Concentration (mg/L)	BOD ₅ /ThOD	BOD ₁₀ /ThOD	BOD ₁₅ /ThOD	BOD ₂₀ /ThOD
3.5	30 ± 8 ^a	53 ± 9	67 ± 11	87 ± 11
7.0	17 ± 2	34 ± 3	52 ± 5	66 ± 4
14.0	13 ± 3	41 ± 5 ^b	62 ± 6	67 ± 5

^a Less than 2 mg/L depleted.

^b Three samples had less than 1 mg/L remaining, not used in subsequent tests (15, 20 days).

fects and growth in these plates was much like that seen in the controls.

CONCLUSIONS

This series of simple tests provided a measure of degradability and microbial toxicity of the FSIIIs. The automatic closed-loop respirometer was the only piece of equipment used that is not readily available in a standard wastewater analysis laboratory. It was used to confirm the closed-bottle BOD test results.

Of these chemicals tested, only DiEGME appeared to meet the criteria of being readily degradable, non-toxic at low concentrations, and toxic enough to be a biostat at higher concentrations. DPG was not readily degradable, although it appeared to begin to degrade after long exposure (> 25 days) to microorganisms. GF showed no signs of biodegradation. Neither DPG nor GF were as potent a biostat as DiEGME.

This series of tests does not eliminate the need for a more thorough examination of the treatability and environmental impact of the final FSII candidates, but this methodology provides a simple means of testing novel chemicals with a minimal outlay of resources when a limited amount of test compound is available.

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